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Circulating immune complexes and C3d in human parasitosis

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The occurrence of glomerulonephritis in schistosomiasis has been documented over the past 10 years [1–4]. The presence of granular deposits of immunoglobulin and complement in the glomeruli suggests that they may be secondary to immune complexes (IC). The formation of IC in the blood can be expected from the simultaneous occurrence in the circulation of parasitic antigens and the corresponding antibodies. A limited number of studies devoted either to human or experimental situations have shown the presence of material behaving as IC in helminthic infections, in some cases by radiolabeled Clq assay, and in others by inhibition of EAC rosette formation [5–7]. In a few patients, indirect evidence has been provided suggesting that schistosomial antigens were present in the IC [7, 8]. In the present study, we have tested the sera of patients suffering from various parasitosis with the sensitive Raji cell radioimmune assay [9]. We chose this method because the immune complex bound to the Raji cell surface is directly accessible to identification of the antigenic moiety. Furthermore, the patients were clinically studied carefully for the presence of nephritis, and biologic evidence of complement activation.

Methods

Patients. A total of 27 patients with schistosomiasis were studied. Thirteen had been infected with *Schistosoma haematobium* and 14 with *Schistosoma mansoni*. The diagnosis was established by the detection of eggs in urine or fecal samples. In some patients, samples were obtained before and after treatment, which consisted of niridazole at a dosage of 25 mg/kg for 7 days.

Twenty-eight patients had filariasis diagnosed by the direct demonstration of microfilariae and serologic techniques [10, 11]. The parasite involved was *Loa-Loa* in 11 cases, *Dracunculus medinensis* in 7 cases, *Wucheria bancrofti* in 7 cases, and *Onchocerca volvulus* in the remaining cases. Treatment, except for *O. volvulus*, relied on notezine for 7 days at conventional dosages. Nineteen patients had hydatidosis diagnosed by pathologic examination of the surgically removed cyst and immunologic methods.

Immune complexes. Detection of immune complexes was performed with the Raji cell radioassay described by Theofilopoulos, Wilson, and Dixon [9] with some modifications. Blood samples were collected and were allowed to clot at 37° C for 1 hour. The sera separated by centrifugation were stored in aliquots at −70° C. Normal human serum samples were obtained in a similar manner from normal blood donors in Hôpital Tenon and in Dakar's transfusion centers. Briefly, 2×10^6 Raji cells were incubated for 30 min at 37° C with 25 μl of 1/4 dilution of the serum to be tested. After three washings, the cells were incubated with an excess of ^{125}I radiolabeled protein A from *Staphylococcus aureus* (Pharmacia Fine Chemical, France) [12]. After three washings, the cell pellets were counted in a gamma counter. The final results were expressed in microgram equivalents of IgG aggregated per milliliter of serum by reference to a standard curve obtained by incubation of known amounts of IgG aggregates in normal human serum.

Complement measurements. For these studies, blood samples were collected in EDTA at a final concentration of 20 mmoles. The plasma was separated by centrifugation and stored in aliquots at −70° C. C3d concentrations were measured with immunoprecipitation techniques as described initially by Perrin, Lambert, and Miescher [13]. Monospecific anti-C3d antiserum was purchased from the

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Netherland Red Cross (Amsterdam, The Netherlands) and used in a unidimensional Laurel technique. The results were expressed in micrograms per milliliter by reference to a standard curve using purified C3d. C3, C4, and properdin were measured by standard radial immunodiffusion methods.

Complement activation by parasitic antigens. By using techniques previously described [10, 11, 14], we prepared crude parasitic antigens from *S. mansoni*, *D. medinensis*, and *O. volvulus* and dissolved them in veronal buffered saline at a concentration of 1 mg/ml. We incubated 100 μl of this solution with 400 μl of normal serum for 30 min at 37° C or 40° C. Complement activation was assessed by measurement of C3 conversion with crossed immunoelectrophoresis and by measurement of C3 consumption by hemolytic assay [15], after incubation with the parasitic antigens from schistosomes and filariae.

Results

Immune complexes. The results are presented in Fig. 1. A limited number of sera were positive in filariasis (36%) and schistosomiasis (24%). The degree of positivity, however, was low, and none of the results of these two groups reached statistical significance when compared to those from a group of normal sera by using the Mann Whitney U test.

C3d levels. The results are presented in Fig. 2. A high percentage of positive results was found both in *S. mansoni* (59%) and *S. haematobium* infection (75%), and in some instances very high levels of C3d could be detected. In filariasis also, and particularly in loasis and lymphatic filariasis, abnormally high levels of C3d were observed. C3, C4, and properdin levels were measured in nine patients who had high C3d levels, and all were normal.

No correlation could be found between the presence of immune complexes and the levels of C3d. Furthermore, neither of these two parameters could be correlated clearly with any clinical or biologic features of the disease, namely hypergammaglobulinemia, eosinophilia, duration, or activity of the disease.

None of the patients with either IC or C3d presented with proteinuria or any other sign of renal disease. Because it is known that therapeutic agents can induce a release of parasitic antigens that may affect immune complexes formation and complement activation, these two parameters were measured in some patients before and after treatment. Figure 3 shows that marked variations of C3d occurred but in a nonsystematic way, because a rise occurred in some patients, whereas there was a fall in the others. The levels of IC, by contrast, did not change clearly, although this is more difficult to evaluate because the amounts of IC detected in the test were small.

Complement activation by parasitic antigens. The results are presented in Table 1. It can be seen that extracts from *S. Mansoni* were inactive, as indicated by the lack of C3 conversion and the absence of any consumption of C3 hemolytic activity. In contrast, extracts from *D. medinensis* produced definite activation of complement indicated by C3 cleavage and C3 consumption. High amounts of *O.*

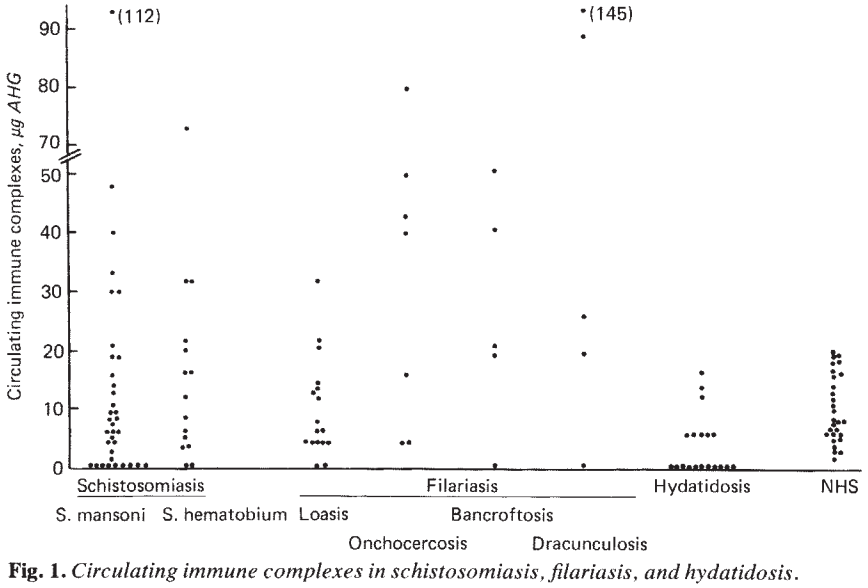

Fig. 1. *Circulating immune complexes in schistosomiasis, filariasis, and hydatidosis.*

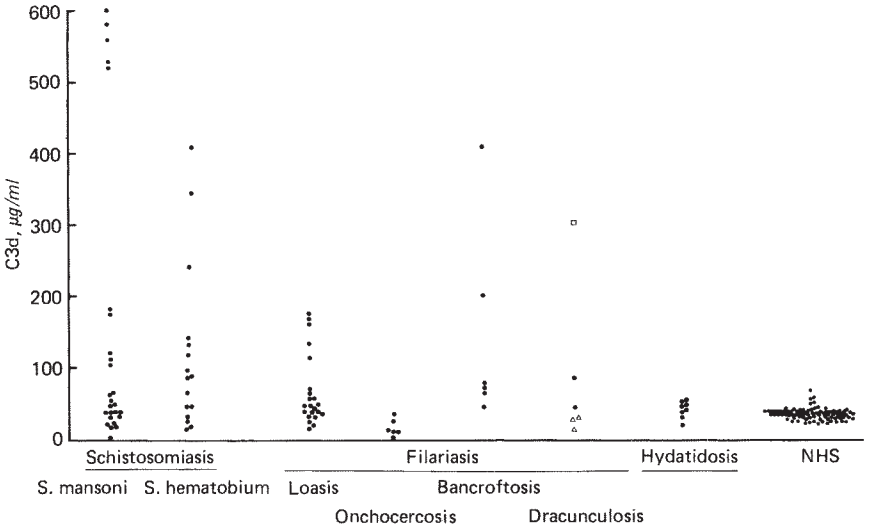

Fig. 2. *C3d levels in schistosomiasis, filariasis, and hydatidosis.*

volvulus extracts produced some complement activation but to a considerably lesser extent.

Discussion

Although parasitosis represents situations in which the occurrence of IC is likely to occur at some stage of the disease, they have not been yet extensively investigated. In schistosomiasis and filariasis, IC have been demonstrated in a variable percentage of patients by the Raji cell assay, the Clq binding assay, and the inhibition of EAC rosettes. The percentage of positive sera in the present series, measured with the Raji cell radioimmune assay, is comparable. The precise composition of the material detected as IC is not known, but recent observations indicate that, at least in some cases, parasitic antigens may be involved [7, 8]. There is, at present, very little evidence to relate the material detected as IC in the serum to the presence of glomerular disease. In the case of *S. haematobium* infection, apart from a single series in Egypt [16], glomerulonephritis has not been clearly established, although IC are found in some cases [5, 17]. Experimental studies also have failed to induce glomerular lesions. In contrast, in *S. Mansoni* infection in human, glomerulonephritis generally associated clinically with liver involvement has been well demonstrated in various studies from Brazil. Histologic examination revealed lesions ranging from a mild glomerulosclerosis to diffuse membranoproliferative glomerulonephritis [1-4]. Granular immunoglobulin and complement could be demonstrated in the glomerular deposits. More recently, parasite

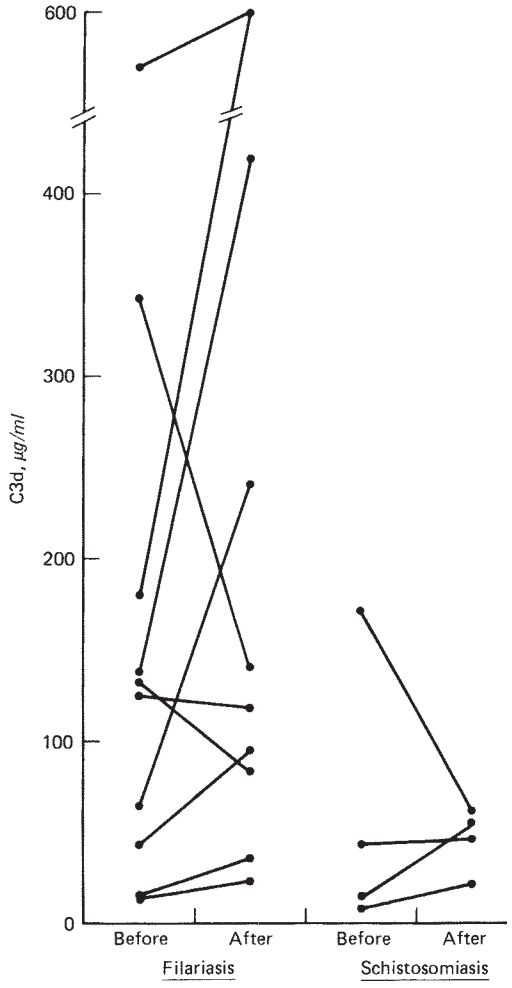

Fig. 3. *Effect of treatment on C3d levels in schistosomiasis and filariasis.*

Table 1. Parasite antigens and complement activation

Antigens, *μg/ml*	% C3 conversion	C3 titration[a] *functional molecules/ml*
None	5	2.3×10^{11}
Dracunculus medinensis		
400	60	9×10^{10}
100	50	1.6×10^{11}
50	40	ND
25	25	2.1×10^{11}
Onchocerca volvulus		
400	10	ND
100	25	1.9×10^{11}
50	20	ND
25	10	2.2×10^{11}
Schistosoma mansoni		
400	5	2.2×10^{11}
100	5	ND
50	5	ND
25	5	2.3×10^{11}

[a] ND means not done.

antigens were found in a limited number of cases [18], and it has been possible to demonstrate specific antischistosome antibody in the glomerular eluates [19]. In experimental animals, infection with *S. Mansoni* has led to the development of glomerulonephritis. In mice [20] it has been possible to demonstrate the presence of parasite antigens and antischistosome antibody in the glomerular deposits. More recent experiments in monkeys [21] suggest that infection with large numbers of parasites can give rise to glomerulonephritis. As in humans, the presence of immunoglobulin and complement could be demonstrated, but it was not possible to detect the presence of schistosome antigen in the glomeruli by immunofluoresence, although it could be demonstrated by counter-immunoelectrophoresis in kidney homogenates [21].

In filariasis, glomerulonephritis has been described very rarely, and no evidence has been provided to link the glomerular lesions to the parasitic infection [22]. In periods following initiation of treatment, serum sickness type reactions may be induced, but glomerulonephritis apparently has not been observed.

Discrepancies between the presence of circulating IC and the absence of glomerulonephritis have been noted previously [23], whereas, conversely, in most instances of primary glomerulonephritis compatible with IC deposition, circulating IC can be detected only with difficulty except for cases of acute proliferative glomerulonephritis. Several hypotheses can be put forward to explain these observations. It is possible that complexes measured by the present techniques are unrelated to immune nephritis, and new technologies may provide further insight [24]. It is also possible, however, that the granular deposits of Ig found in the glomeruli are not secondary to the deposition of IC formed in the blood but rather to the local formation of IC through binding of a circulating antibody to an antigen previously deposited in the glomeruli. A number of models of this type have recently been described [25, 26]. Experimental evidence with *S. mansoni* infection in baboons actually suggests that such a mechanism could be operative because schistosome antigens can be detected in cortex extracts before glomerular deposition of Ig [21].

It is interesting to note that whereas low levels of IC were found, C3 fragments were detected very frequently in abnormally high amounts. This lack of correlation suggested that parasitic antigens, which are probably very abundant in vivo (and in some instances may circulate within the blood stream), could be directly responsible for complement activation. We could not support this hypothesis because the parasitic antigen preparations that we used were unable to activate complement in vitro except for *D. medinensis*, which is responsible for a parasitosis in which we could not demonstrate C3d in vivo. This may be due to the very localized lifecycle of this worm. Alternatively, it could be proposed that IC are indeed formed but remain localized in the extravascular space or bound to fixed structures. Direct complement activation by parasite antigens cannot be ruled out, however, because the preparation of antigens used in vitro may not contain the potentially active fractions. In schistosomiasis, a wide variety of antigens have been identified [27], and only some of them may be active. Furthermore, in vivo, one should consider also the possibility that the antigens may change in structure, either by a mechanism of adaptation to the host [14] or by a variation of their surface, as has been shown in trypanosomiasis [28]. Whatever the cause of the complement activation, it did not induce hypocomplementemia because levels of C3, C4, and properdin in nine patients who had high C3 values were within normal limits. The observations should be considered in the light of previous studies based on turnover of C3 in hypocomplementemic diseases, which show that an increased catabolism is almost invariably associated with decreased synthesis [29]. On the other hand, previous work indicates that complement synthesis in inflammatory conditions, as in some instances of liver disease (which are likely to occur in some of our patients), may be increased [30, 31]. In some instances of

chronic active hepatitis, C3 split products may be found in the serum with concomitant increased C levels [32]. The results obtained in our patients may therefore be explained by hypercatabolism occurring in patients with increased synthesis. Studies along this line are at present in progress.

In conclusion, further work is required to establish the relationship between the immunologic abnormalities found in parasitic infections and the occurrence of glomerulonephritis. Studies on the metabolism of parasite antigens and the immunologic response that they induce will provide new insights.

Summary

Using the Raji cell radioimmune assay, we found low levels of circulating immune complexes (IC) in a small percentage of patients with schistosomiasis and filariasis. C3d levels, measured by immunoprecipitation, were elevated in a large number of these patients, whereas complement levels were within normal limits. Proteinuria was not found in any of the 55 patients studied. Circulating IC or elevated C3d levels were not found in any of the 19 patients with hydatidosis. The increased C3d levels, apparently not related to circulating IC, may be due to direct complement activation by parasite antigens or to sequestered IC. The latter hypothesis appears more attractive because the highest levels of C3d were found in schistosomiasis whereas schistosome antigens were unable to activate complement in vitro.

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